European Journal of Medicinal Chemistry 157 (2018) 1115–1126

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Conjugation of a 5-nitrofuran-2-oyl moiety to aminoalkylimidazoles produces non-toxic nitrofurans that are efficacious *in vitro* and *in vivo* against multidrug-resistant *Mycobacterium tuberculosis*

Mikhail Krasavin [a, *], Alexei Lukin [b], Tatiana Vedekhina [b], Olga Manicheva [c], Marine Dogonadze [c], Tatiana Vinogradova [c], Natalia Zabolotnykh [c], Elizaveta Rogacheva [d], Liudmila Kraeva [d], Piotr Yablonsky [c]

[a] Saint Petersburg State University, Saint Petersburg, 199034, Russian Federation
[b] Lomonosov Institute of Fine Chemical Technologies, MIREA – Russian Technological University, Moscow, 119571, Russian Federation
[c] Saint Petersburg Research Institute of Phthisiopulmonology, 2-4 Ligovsky Prospekt, Saint Petersburg, 191036, Russian Federation
[d] Pasteur Institute of Epidemiology and Microbiology, 14 Mira Street, Saint Petersburg, 197101, Russian Federation

ARTICLE INFO

Article history:
Received 10 July 2018
Received in revised form
22 August 2018
Accepted 25 August 2018
Available online 29 August 2018

Keywords:
Chimera drug design
Nitrofurans
Imidazoles
Antitubercular
Mycobacterium tuberculosis
Multidrug resistant
Selective antimycobacterial activity
Druglikeness

ABSTRACT

Within the general nitrofuran carboxamide chemotype, chimera derivatives incorporating diversely substituted imidazoles attached via an alkylamino linker were synthesized. Antimycobacterial evaluation against drug-sensitive *M. tuberculosis* H37Rv strain identified five active druglike compounds which were further profiled against patient-derived *M. tuberculosis* strains *in vitro*. One of the compounds displayed promising potent activity (MIC 0.8 μg/mL) against one of such strains otherwise resistant to such first- and second-line TB therapies as streptomycin, isoniazid, rifampicin, ethambutol, kanamycin, ethionamide, capreomycin and amikacin. The compound was shown to possess low toxicity for mice ($LD_{50} = 900.0 \pm 83.96$ mg/kg) and to be similarly efficacious to etambutol, in the mouse model of drug-sensitive tuberculosis, and to neurotoxic cycloserine in mice infected with MDR tuberculosis.

1. Introduction

The constant emergence of drug-resistant microorganisms [1] mandates that new molecular entities are brought up through the drug discovery process into preclinical and clinical development. However, the current situation with serious non-contained infections in developing countries such as tuberculosis (caused by *Mycobacterium tuberculosis* or *MTb*) is such that the demand for new drug candidates in the development pipeline is not met with adequate productivity in the discovery process [2]. The multidrug-resistant (MDR) forms of tuberculosis are particularly hard to treat with the existing therapies, which leads to increased death rates, especially from co-infection with HIV [3]. The latest breakthrough in antitubercular drug development productivity (which manifested itself in the approvals of Janssen's bedaquiline [4] and Otsuka's delamanid [5]) is somewhat reassuring. However, other classes of drugs should be validated for the development, even by revisiting known antimicrobial chemotypes and attempting to optimize them specifically to target *MTb*.

Nitrofurans (along with nitroimidazoles to which delamanid belongs) are the so-called bioreducible compounds that have demonstrated potential to treat various infectious diseases caused by bacterial pathogens [6]. Their mechanism of action is thought to involve reduction, by the bacterial cell wall enzyme, of the nitro-group to produce free radical species that can react with the bacterial biomolecules and are, therefore, lethal to the microorganism [7]. The major concern associated with nitrofurans and possibly hindering their advancement into development is their being also a structural alert from toxicology perspective. Indeed, if it is not

* Corresponding author. Laboratory of Chemical Pharmacology, Saint Petersburg State University, 26 Universitetskyi Prospect, Peterhof, 198504, Russian Federation.
E-mail address: m.krasavin@spbu.ru (M. Krasavin).

https://doi.org/10.1016/j.ejmech.2018.08.068
0223-5234/

ensured that a chemotherapeutic agent in question is selectively taken up and metabolized by the bacteria, issues of toxicity and mutagenicity may arise [8,9]. It is perhaps unsurprising that since the introduction of nitrofurazone (**1**) [10] in the 1940s as well as the other, mostly topical, compounds nifuroxazide (**2**) and nitrofurantoin (**3**) [11] examples of nitrofuran compounds as advanced as preclinical phase of development have remained scarce. However, the recent success of nitroimidazoles delamanid (*vide infra*) and pretomanid (which is currently in phase III clinical study [12]) led to a belief that the nitrofuran drug class, too, can deliver efficacious and non-toxic antitubercular drug candidates via a careful optimization of compounds' periphery. The interest to revisiting this well-established class of antimicrobial agents in antitubercular context (i. e. in the area highly prone to drug resistance issues) is additionally fueled by the latest clinical data meta-analysis on furantoin demonstrating that the potential of microbial resistance to nitrofurans is low [13].

Exploration of the nitrofuran periphery for the purposes of selectively increasing antimycobacterial activity and reducing toxicity to the host is potentially a vast and resource-intensive area of research. Here certain guidelines for selecting specific groups and moieties should be considered. While predictive computational models are being developed [14], medicinal chemist often choose a more intuitive drug design strategy whereby the pharmacophoric nitrofuran moiety is conjugated with or embedded into structures that also have some documented antitubercular efficacy associated with them. For instance, nitrofuranylamides discovered by Lee in the form of the early hit (**4**) [15] and optimized into more advanced compounds such as Lee562 (**5**) with demonstrated safety and efficacy *in vivo* [16] attest both to acceptability of the amide linkage and the presence of basic heterocyclic moieties in the candidate compounds' periphery. *N*-Aminolactams conjugated with a nitrofuran motif via a hydrazone linkage (**6**) helped reveal a preferred *N*-cyclohexyl carboxamide periphery [17]. Nitrofuran-containing isostere **7** of antimycobacterial natural product (+)-calanolide A illustrates another productive design strategy [18]. Merging a broadly antimicrobial 2-aminothiazole and antimycobacterial nitrofuranyl moieties in the structure of compound **8** speaks for the power of the chimera design [19].

In this work, we explored a similar approach with respect to combining, within a single molecule (**9** or **10**), the pharmacophoric 5-nitrofuran-2-oyl moiety and a substituted imidazole motif linked together by a diverse set of aminoalkyl linkers (Fig. 1). This research was particularly motivated by the recently established significance of non-nitrated imidazole motifs in the antitubercular compound design [20] and the validation of imidazole fragments in targeting *MTb* [21]. Herein, we describe the synthesis of compounds **9**–**10** (via $Zn(OTf)_2$-catalyzed amination-cyclization of propargylamides followed by the introduction of the 5-nitrofuran-2-oyl moiety) and the results of their profiling, as potential antitubercular agents *in vitro* and *in vivo*.

2. Results and discussion

2.1. Chemistry

A variety of substituted imidazoles **14** or **15** containing the aminoalkyl side chains required for conjugation to 5-nitrofur-2-yl moiety via an amide linkage in position 2 or 1 of the imidazole nucleus were accessed from the respective propagylamides **12** (prepared, in turn, from carboxylic acids **11** in good to excellent yield, *vide infra*) according to $Zn(OTf)_2$-catalyzed amination-cyclization sequence as described by Beller [22] and us [23]. The initial imidazole cycloadducts **13** (although observed by TLC and ^1H NMR analysis of the reaction mixtures) were not isolated and the crude material obtained in these reactions was taken on to the deprotection step. The latter was brought about by 4 N solution of HCl in 1,4-dioxane and the desired 2- and 1-aminoalkyl substituted imidazoles **14** and **15**, respectively, were obtained as di- and trihydrochloride salts (see Experimental Section) in moderate to good yields over two steps (see Table 1). The amine hydrochlorides **14** or **15**, in presence of triethylamine (required to obtain the free-base amine for acylation) was brought in contact with acyl imidazolide prepared *in situ* from 5-nitrofuran-2-carboxylic acid. The yield in the last acylation step obtained via the use of CDI was surprisingly low throughout the range of amines **14** and **15** employed. However, the employment of an alternative acylation strategy *via in situ* preparation of 5-nitrofur-2-oyl chloride or the use of EDC in lieu of CDI (as was described previously for the same type of acylation reaction by Lee and co-workers [24]) did not improve the yield of the reaction. Overall, the target conjugates of a substituted imidazole moiety with pharmacophoric 5-nitrofur-2-yl moiety via an aminoalkyl linker **9** and **10** were obtained in four chemical operations involving only three isolation or purification steps (Scheme 1).

2.2. In vitro biological activity

The antimycobacterial activity of compounds **9a-e** and **10a-g** was initially evaluated against the drug-sensitive H37Rv strain of *MTb*. As it is evident from the data presented in Table 1, substantial antimycobacterial activity was observed for compounds belonging to either series. It is obvious that, to a large degree, introducing various cyclic motifs as rigidity elements into the structure of the linker appeared to increase the antimycobacterial potency.

Five compounds (**9a-b**, **9d**, **10d** and **10e**) that displayed the lowest values of minimum inhibitory concentration (MIC) were analyzed for the critical characteristics determining druglikeness and prospects for sufficiently high oral bioavailability [25]. As it is evident from the calculated data summarized in Table 2, the compounds which displayed the highest antimycobacterial activity, are well within the limits of druglikeness (as defined by the Lipinski's rule-of-five [26]). The notable feature of the five active leads identified in this work is the narrow range of calculated total polar surface area (TPSA) these compounds fall into. This is in line with the significance of TPSA characteristic for bacterial cell wall permeability noted by Tan and co-workers [27].

The five compound selected based on their antimycobacterial activity against H37Rv strain were further evaluated for the same activity in MDR strains available in the tuberculosis patient-derived mycobacterial strain collection of Saint Petersburg Research Institute of Phthisiopulmonology. In general, the activity of these compounds against the MDR strains was comparable to or significantly lower than that observed on H37Rv strain. The notable exception was compound **9d** which displayed even somewhat higher activity (compared to drug-sensitive strain) against 2712 strain which was isolated from a patient not responding to a number of first- and second-line drugs (Table 3).

It should be noted that the most active compound (**9d**) characterized herein further (*vide infra*) carries a number of attractive features (a rigid achiral azetidine linker, low lipophility) which makes the compound a promising drug development candidate [28]. The same compound did not display any activity (Table 4) against three (*E. faecium, K. pneumoniae and P. aeruginosa*) out of six Gram-positive and Gram-negative bacteria belonging to the so-called ESKAPE panel of pathogens (i. e. the ones most prone to developing resistance to drugs) [29]. The activity against the other three ESKAPE pathogens (*S. aureus*, *A. baumanii* and *E. aerogenes*) can be noted albeit it was lower than the activity of ciprofloxacin (employed as a positive control).

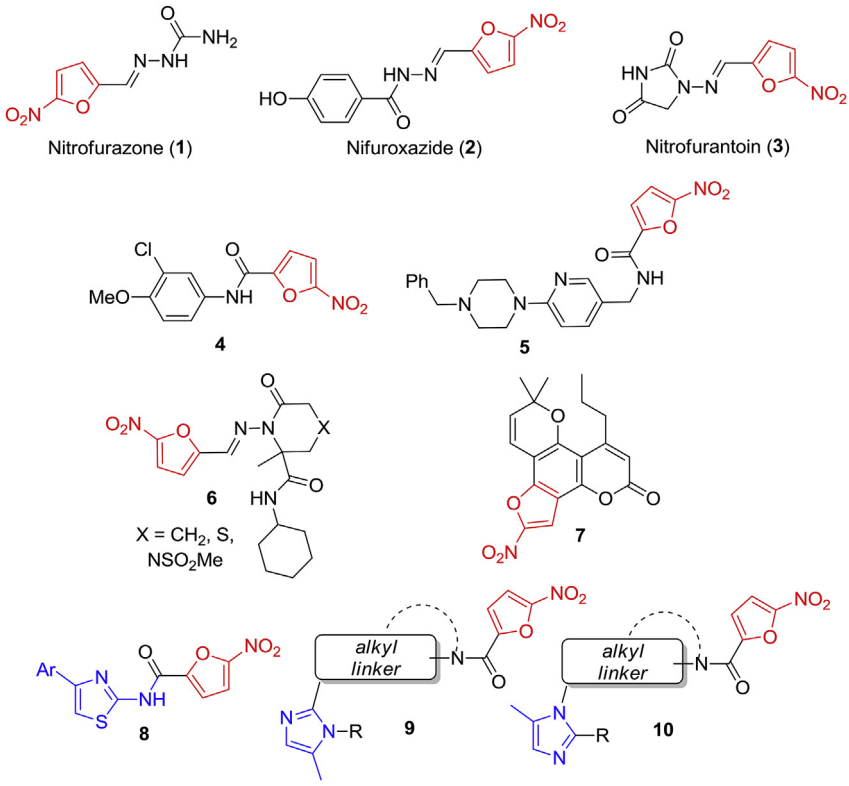


Fig. 1. Known antibacterial (**1**–**3**) and antimycobacterial (**4**–**8**) nitrofurans and the general structure of compounds **9**–**10** investigated in this work.

2.3. *In vivo* toxicology evaluation

The toxicology data were obtained for the frontrunner compound (**9d**) identified in this study as described in Section 4.4. Their analysis is presented in Table 5. The toxicometry assessment along with necropsy data and observation of the animals during the period of up to 24 h post intoxication allowed concluding that compound **9d** belongs to the low toxicity group (class 4) according to classification by Hodge and Sterner [30].

2.4. *In vivo* efficacy testing

Having established the low toxicity profile of compound **9d** in mice, we were interested to see if the same compound would demonstrate efficacy lowering infection symptoms in mice infected with tuberculosis. Oral administration of compound **9d** (20 mg/kg) to mice infected with drug-sensitive H37Rv strain of *Mtb* over 42 days significantly reduced the key *in vivo* indicators of severity of tubercular infection compared to no-treatment group (see Section 4.5 for experimental details and ESI — for statistical analysis). This effect found to be practically identical to that produced by etambutol 20 mg/kg p. o. (Fig. 2).

The established oral efficacy of compound **9d** in alleviating symptoms of drug-sensitive tuberculosis in mice encouraged us to consider it for possible replacement of the drug components in the four-component treatment scheme which includes prothionamide (Pt), levofloxacin (Lfx), *p*-aminosalicylic acid (PAS) and cycloserine (Cs) [31] and to which the "2712" strain employed earlier for *in vitro* testing (*vide supra*) was sensitive. It should be noted that it had been demonstrated by drug susceptibility testing [32] that each component in this four-component [33] treatment scheme is critical for its efficacy. However, it is of paramount importance to identify possible replacements for the components of this drug combination should patients exhibit adverse effects in response to a particular component or if resistance to any of them emerges. The recent reports on emerging resistance to Cs [34] as well as its well-documented neurotoxicity [35] prompted us to consider Cs as a good candidate for component replacement in the above four-drug combination. To our delight, the use of compound **9d** (given orally) in lieu of Cs produced a new drug combination which was similarly efficacious to the original four-component combination in alleviating symptoms of experimental tuberculosis in mice infected by multidrug-resistant "2712" strain (Fig. 3).

3. Conclusion

By employing a chimera drug design strategy - i. e. combining the pharmacophoric antibacterial nitrofuran moiety with structurally diverse aminoalkyl imidazole fragments present in various reported antitubercular drugs — we have identified a lead compound that demonstrated high potency (MIC 1.6 μg/mL) against drug-sensitive *Mtb* H37Rv strain *in vitro*. The same compound was found to have comparable activity *in vitro* against three multidrug-resistant strains from the pathogenic mycobacterial strain collection of Saint Petersburg Research Institute of Phthisiopulmonology. The major safety concern associated with the development of bioreducible nitrofuran drugs — namely, their non-specific toxicity — has been eliminated by *in vivo* toxicity testing in mice where the lead compound demonstrated low toxicity profile. Finally, the same compound proved similarly efficacious to the clinically used etambutol in treating tuberculosis in mice infected with drug-sensitive H37Rv strain. It also showed similar activity to neurotoxic cycloserine (emerging resistance to which has been recently reported) when employed as a part of a four-drug combination therapy. These results demonstrate that the lead compound identified and characterized in this study represents a promising candidate for further development.

Table 1
Structures, yields and *in vitro* antimycobacterial activity of compounds **9a-e** and **10a-g** against drug-sensitive strain H37Rv of *MTb* investigated in this work.

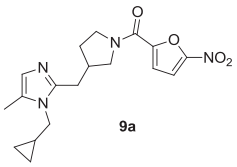
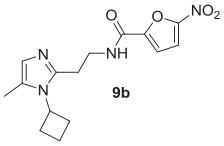
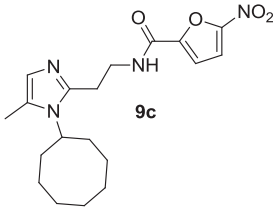
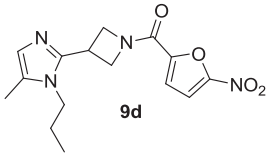
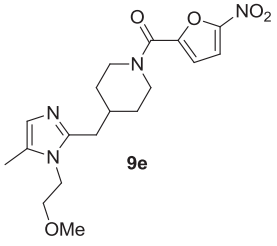
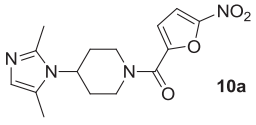
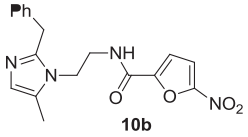
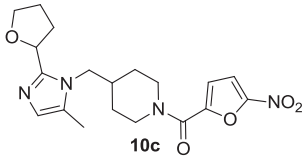
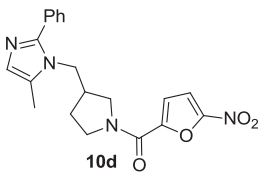
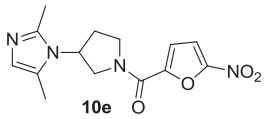
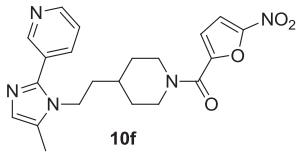
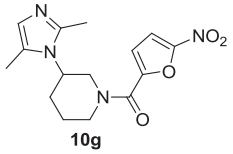
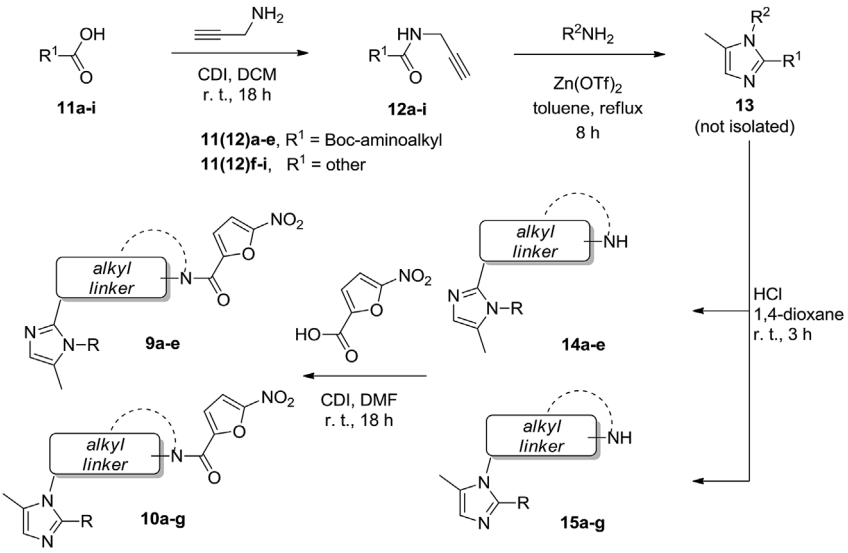
Entry	Compound	Yield of 9 (10), %[a]	12 (% yield)	14 (15) (% yield)[b]	MIC (μg/mL)
1	 **9a**	66	**12a** (88)	**14a** (55)	6.2
2	 **9b**	64	**12b** (97)	**14b** (47)	6.2
3	 **9c**	48	**12b** (97)	**14c** (36)	25
4	 **9d**	42	**12c** (92)	**14d** (44)	1.6
5	 **9e**	21	**12d** (92)	**14e** (72)	>100
6	 **10a**	47	**12e** (54)	**15a** (46)	>100
7	 **10b**	62	**12f** (92)	**15b** (42)	50
8	 **10c**	28	**12g** (85)	**15c** (44)	50
9	 **10d**	48	**12h** (98)	**15d** (42)	6.2

Table 1 (*continued*)

Entry	Compound	Yield of 9 (10), %[a]	12 (% yield)	14 (15) (% yield)[b]	MIC (μg/mL)
10	 **10e**	57	**12e** (54)	**15e** (34)	3.1
11	 **10f**	38	**12i** (81)	**15f** (41)	100
12	 **10g**	49	**12e** (54)	**15g** (41)	100

[a] Isolated yield of **9** (**10**) from **14** (**15**).
[b] Isolated yield of **14** (**15**) over 2 steps from **12**.



Scheme 1. Synthesis of compounds **9a-e** and **10a-g** investigated in this work.

Table 2
Molecular characteristics of selected compounds **9** and **10** defining their druglikeness and oral bioavailability.

Molecular characteristic	9a	9 b	9 d	10 d	10e
Molecular weight	358.40	318.33	318.33	380.40	304.31
cLogP[a]	2.28	1.32	1.43	3.24	0.83
HBD/HBA	0/8	1/8	0/8	0/8	0/8
# rotatable bonds	6	6	5	5	3
TPSA, Å^2[a]	97.10	105.89	97.10	97.10	97.10

[a] Calculated using online molecular property calculator available at www.molinspiration.com.

4. Experimental protocols

All reactions were conducted in oven-dried glassware in atmosphere of nitrogen. Melting points were measured with a Buchi B-520 melting point apparatus and were not corrected. Analytical thin-layer chromatography was carried out on Silufol UV-254 silica gel plates using appropriate mixtures of ethyl acetate and hexane. Compounds were visualized with short-wavelength UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker MSL-300 spectrometers in DMSO-d_6 using TMS as an internal standard. Mass spectra were recorded using Shimadzu LCMS-2020 system with electron impact (EI) ionization. All and reagents and solvents were obtained from commercial sources and used without purification.

4.1. Synthesis

4.1.1. General procedure for the preparation of propargylamides **12a-i** (exemplified by the synthesis of acetic acid propargylamide **12e**)

To a solution of acetic acid (4.0 g, 67 mmol) in CH2Cl2 (100 mL) *N,N*-carbonyldiimidazole (CDI, 11.88 g, 73 mmol) was

Table 3
Antimicrobial activity of selected compounds **9** and **10** evaluated against mutant MDR clinical isolates of *M. Tuberculosis*.

Mutant strain	Gene mutations				*MTb* family	MDR profile[a]	MIC (μg/mL)				
	rpoB	*katG*	*inhA*	*ahpC*			9a	9 b	9 d	10 d	10e
2712[b]	S531L	I335V	–	T10	Beijin	SHREKCapA	1.6	12.5	0.8	3.1	25
5023[c]	S531L	S315T	*wt*	*wt*	Beijin	SHREK	6.2	12.5	3.1	6.2	25
7106[b]	S531L	S315T	*wt*	*wt*	Beijin	SHREKCapAZ	12.5	12.5	3.1	6.2	12.5

[a] S – streptomycin, H – isoniazid, R – rifampicin, E – ethambutol, K – kanamycin, Et – ethionamide, Cap – capreomycin, A – amikacin, Z – pyrazinamide.
[b] Pulmonary tuberculosis.
[c] Bone and joint tuberculosis.

Table 4
Antimicrobial activity of the lead compound (**9d**) against common Gram-positive and Gram-negative pathogens evaluated by the Kirby-Bauer disk diffusion method.

	Enterococcus faecium	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Acinetobacter baumannii*	*Pseudomonas aeruginosa*	*Enterobacter aerogenes*
9d	0	15	0	25	0	19
cyprofloxacin	25	27	26	31	25	26

[a] Data shown are the diameter (mm) of bacterial growth inhibition zone around the circular area of dried compound **9d** solution or disc containing a standard amount of ciprofloxacin.

Table 5
Toxicology data obtained on oral administration of compound **9d** to C56BL6 mice.

Dose (mg/kg)	Dead/total animals	z[a]	d[b]	zd	LD_{50}	LD_{16}	LD_{84}	LD_{100}
1100	6/6	–	–	–	900.0 ± 83.96[c]	287.5 ± 83.96[c]	1037.5 ± 83.96[c]	1100.0 ± 83.96[c]
1000	5/6	5.5	100	550				
900	3/6	4.0	100	400				
800	1/6	2.0	100	200				
700	0/6	0.5	100	50				
Σ				1200				

[a] 50% of the animals killed by the two subsequent doses.
[b] Interval between two subsequent doses.
[c] SEM = sqrt (k·s·d/n) = sqrt (0.564·750.0·100/6) = 83.96, where s = LD_{84} - LD_{16} = 750.0.

added and the mixture was stirred at r. t. for 30 min. Propargylamine (6.75 g, 73 mmol) was added dropwise and the stirring continued for 18 h. The reaction mixture was successively washed with 5% aqueous citric acid (2 × 30 mL) and 10% aqueous K2CO3 (2 × 30 mL). The organic phase was dried over anhydrous Na2SO4, filtered, concentrated *in vacuo* and further dried under high vacuum to provide analytically pure **12e** (54%) as pale yellow solid: m. p. 70–72°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.33 (br.s, 1H), 3.83 (dd, J = 5.5, 2.5 Hz, 2H), 3.08–3.02 (m, 1H), 1.82 (s, 3H); MS *m/z* 98.1 ($M+H^+$).

*4.1.1.1. (3-N-tert-Butoxycarbonylpyrrolidyl)acetic acid propargylamide (**12a**).* Yellow oil, yield 88%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.28 (t, J = 5.1 Hz, 1H), 3.84 (dd, J = 5.1, 2.2 Hz, 2H), 3.42–3.38 (m, 1H), 3.34–3.25 (m, 1H), 3.18–3.11 (m, 1H), 3.02–2.99 (m, 1H), 2.86–2.77 (m, 1H), 2.45–2.35 (m, 1H), 2.20–2.15 (m, 2H), 1.97–1.85 (m, 1H), 1.54–1.45 (m, 1H), 1.37 (s, 9H); MS *m/z* 267.3 ($M+H^+$).

*4.1.1.2. 3-(N-tert-Butoxycarbonylamino)propanoic acid propargylamide (**12b**).* Pale yellow solid, m. p. 111–113°C, yield 97%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.22 (d, J = 3.5 Hz, 1H), 6.64 (br.s, 1H), 3.83 (d, J = 3.1 Hz, 2H), 3.12 (dd, J = 13.3, 6.8 Hz, 2H), 3.03 (s, 1H), 2.25 (t, J = 7.2 Hz, 2H), 1.37 (s, 9H); MS *m/z* 227.3 ($M+H^+$).

*4.1.1.3. 1-tert-Butoxycarbonylazetidine-3-carboxylic acid propargylamide (**12c**).* Pale yellow solid, m. p. 107–109°C, yield 92%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.47 (t, J = 4.9 Hz, 1H), 3.96–3.77 (m, 6H), 3.30–3.20 (m, 1H), 3.14 (t, J = 2.5 Hz, 1H), 1.37 (s, 9H); MS *m/z* 239.3 ($M+H^+$).

*4.1.1.4. 1-tert-Butoxycarbonyl-4-piperidylacetic acid propargylamide (**12d**).* Pale yellow solid, m. p. 90–92°C, yield 92%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.17 (s, 1H), 3.93–3.82 (m, 4H), 3.00–2.96 (m, 1H), 2.68 (t, J = 11.9 Hz, 2H), 2.05–1.99 (m, 2H), 1.90–1.77 (m, 1H), 1.62–1.52 (m, 2H), 1.38 (s, 9H), 1.08–0.91 (m, 2H); MS *m/z* 281.4 ($M+H^+$).

*4.1.1.5. Phenylacetic acid propargylamide (**12f**).* Pale yellow solid, m. p. 80–82°C, yield 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.42–7.24 (m, 5H), 5.74 (s, 1H), 4.02 (dd, J_1 = 5.3 Hz, J_2 = 2.5 Hz, 2H), 3.61 (s, 2H), 2.20 (t, J = 2.5 Hz, 1H); MS *m/z* 174.2 ($M+H^+$).

*4.1.1.6. Tetrahydro-2-furoic acid propargylamide (**12g**).* Pale yellow solid, m. p. 54–56°C, yield 85%; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.88 (br.s, 1H), 4.38 (dd, J = 8.3, 5.8 Hz, 1H), 4.09–4.05 (m, 2H), 4.01–3.86 (m, 2H), 2.36–2.23 (m, 2H), 2.14–2.02 (m, 1H), 2.00–1.83 (m, 2H); MS *m/z* 154.2 ($M+H^+$).

*4.1.1.7. Benzoic acid propargylamide (**12h**).* Pale yellow solid, m. p. 103–105°C, yield 98%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.90 (s, 1H, NH), 7.86 (d, J = 7.2 Hz, 2H, Ph 2,6-H), 7.59–7.41 (m, 3H, Ph 3,4,5-H), 4.06 (dd, J_1 = 5.5 Hz, J_2 = 2.4 Hz, 2H, CH_2), 3.10 (s, 1H, CH); MS *m/z* 160.2 ($M+H^+$).

*4.1.1.8. Nicotinic acid propargylamide (**12i**).* Pale yellow solid, m. p. 85–87°C, yield 81%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm δ 9.15 (s, 1H, NH), 9.00 (d, J = 1.7 Hz, 1H, Py 2-H), 8.71 (dd, J_1 = 4.7 Hz, J_2 = 1.3 Hz, 1H, Py 6-H), 8.25–8.15 (m, 1H, Py 4-H), 7.51 (dd, J_1 = 7.8 Hz, J_2 = 4.8 Hz, 1H, Py 5-H), 4.08 (dd, J_1 = 5.4 Hz, J_2 = 2.4 Hz, 2H, CH_2), 3.16 (t, J = 2.3 Hz, 1H, CH); MS *m/z* 161.2 ($M+H^+$).

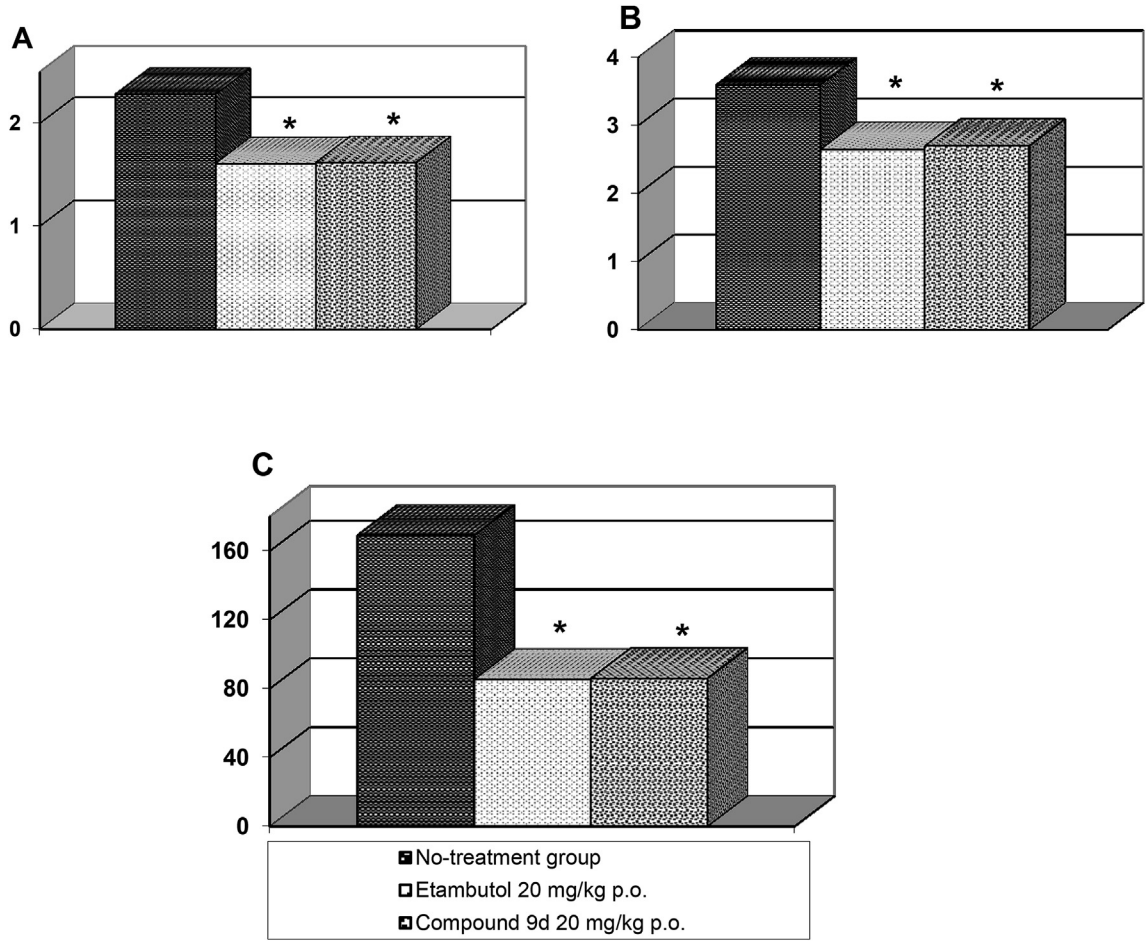


Fig. 2. *In vivo* indicators of antitubercular efficacy of compound **9d** in comparison with etambutol in mice infected with drug-sensitive H37Rv strain.
[a] A: Lung mass index (relative units); B: Tubercular foci index (relative units); C: cfu per lung mass unit.

*4.1.2. General procedure for the preparation of compounds **14a-e** and **15a-g** exemplified by the synthesis of 2,5-dimethyl-1-(4-piperidyl)-1H-imidazole dihydrochloride (**15a**)*

To a solution of **12e** (2.0 g, 21 mmol) in toluene (50 mL) *tert*-butyl 4-aminopiperidine-1-carboxylate (4.9 g, 6.25 mmol) and Zn(OTf)2 (1.6 g, 4.5 mmol) were added. The resulting mixture was heated at reflux for 8 h and then cooled down to r. t. It was poured into 10% aqueous K2CO3 (40 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over anhydrous Na2SO4, filtered and concentrated *in vacuo*. The residue was fractionated by column chromatography on silica gel using 0 → 2.5% gradient of methanol in chloroform to give, on evaporation of the volatiles, orange oil. It was dissolved in 1,4-dioxane (10 mL) and 4 M HCl in 1,4-dioxane (3 mL, 12.0 mmol) was added. The mixture was stirred at r. t. and the brown crystalline precipitate was collected by filtration. Yield 46%, m. p. 194–196°C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.58 (s, 1H), 9.76 (d, J = 9.4 Hz, 1H), 9.47 (d, J = 8.1 Hz, 1H), 7.32 (s, 1H), 4.76–4.62 (m, 1H), 3.41–3.31 (m, 2H), 3.20–3.03 (m, 2H), 2.72 (s, 3H), 2.60–2.52 (m, 1H), 2.38 (s, 3H), 2.13–2.02 (m, 2H), 1.89–1.52 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 144.5, 130.3, 116.0, 52.8, 43.0, 26.4, 12.6, 11.3; MS *m/z* 180.3 (M+H$^+$).

*4.1.2.1. 1-(Cyclopropylmethyl)-5-methyl-2-(3-pirrolidylmethyl)-1H-imidazole dihydrochloride (**14a**).* Orange oil, yield 55%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.85 (br.s, 1H), 9.90 (br.s, 1H), 9.73 (br.s, 1H), 7.41 (2s, 1H), 4.18–4.01 (m, 2H), 3.34–3.20 (m, 4H), 3.14–3.03 (m, 1H), 2.97–2.78 (m, 2H), 2.32 (2s, 3H), 2.09–1.97 (m, 1H), 1.72–1.58 (m, 1H), 1.26–1.12 (m, 1H), 0.58–0.51 (m, 2H), 0.49–0.43 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 144.7, 130.7, 116.1, 48.8, 48.6, 44.3, 36.0, 29.6, 27.4, 11.2, 9.9, 4.5, 4.4; MS *m/z* 220.3 (M+H$^+$).

*4.1.2.2. 2-(2-Aminoethyl)-1-cyclobutyl-5-methyl-1H-imidazole dihydrochloride (**14b**).* Pale gray solid, m. p. 218–220°C, yield 47%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.86 (br.s, 1H), 8.72 (br.s, 3H), 7.37 (s, 1H), 5.07–4.93 (m, 1H), 3.44 (t, J = 7.5 Hz, 2H), 3.30–3.19 (m, 2H), 2.69–2.53 (m, 4H), 2.33 (s, 3H), 1.92–1.74 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 143.0, 131.2, 116.7, 51.4, 36.8, 30.1, 23.9, 15.5, 11.3; MS *m/z* 180.3 (M+H$^+$).

*4.1.2.3. 2-(2-Aminoethyl)-1-cyclooctyl-5-methyl-1H-imidazole dihydrochloride (**14c**).* Pale gray solid, m. p. 177–179°C, yield 36%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.75 (br.s, 1H), 8.65 (br.s, 3H), 7.39 (s, 1H), 4.44 (br.s, 1H), 3.52–3.44 (m, 2H), 3.32–3.16 (m, 2H), 3.01–2.88 (m, 1H), 2.56–2.51 (m, 1H), 2.33 (s, 3H), 2.18–2.08 (m, 2H), 1.96–1.84 (m, 2H), 1.80–1.63 (m, 6H), 1.55–1.44 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.6, 142.1, 129.9, 81.3, 73.5, 36.9, 35.5, 33.4, 32.3, 28.2, 25.8, 25.5, 25.2, 11.7; MS *m/z* 236.4 (M+H$^+$).

*4.1.2.4. 2-(3-Azetidyl)-5-methyl-1-propyl-1H-imidazole difluoroacetate (**14d**).* Dark brown oil, yield 44%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.64 (br.s, 1H), 9.09 (br.s, 1H), 7.51 (s, 1H), 4.72–4.58 (m, 1H), 4.47–4.38 (m, 2H), 4.35–4.27 (m, 2H),

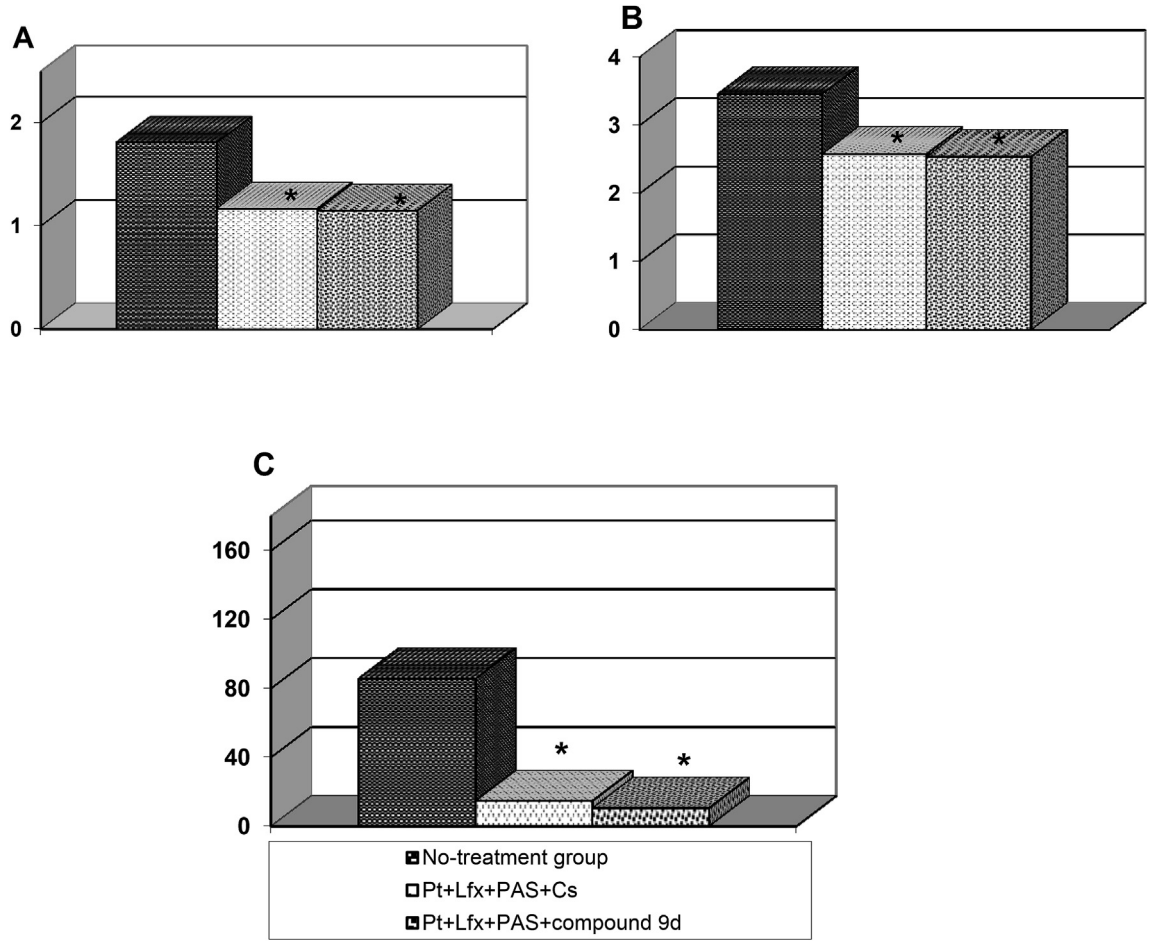


Fig. 3. *In vivo* indicators[a] of antitubercular efficacy of the combination therapy of prothionamide (Pt), levofloxacin (Lfx), *p*-aminosalycilic acid (PAS) and cycloserine (Cs) in comparison with the combination Pt + Lfx + PAS + compound **9d** (replacing Cs) in mice infected with multidrug-resistant "2712" strain.
[a] A: Lung mass index (relative units); B: Tubercular foci index (relative units); C: cfu per lung mass unit.

4.02–3.90 (m, 2H), 2.28 (s, 3H), 1.69–1.55 (m, 2H), 0.89 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 159.3 (q, $J = 36.7$ Hz), 143.9, 131.1, 116.7, 116.0 (q, $J = 290.6$ Hz), 49.4, 46.1, 27.2, 22.9, 10.7, 9.0; MS *m/z* 180.3 ($M+H^+$).

*4.1.2.5. 1-(2-Methoxyethyl)-5-methyl-2-(4-piperidylmethyl)-1H-imidazole dihydrochloride (**14e**).* Dark brown solid, m. p. 124–126°C, yield 72%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.93 (br.s, 1H), 9.48 (br.s, 1H), 9.25 (br.s, 1H), 7.37 (s, 1H), 4.30 (t, $J = 4.9$ Hz, 2H), 3.60 (t, $J = 4.8$ Hz, 2H), 3.23 (s, 3H), 3.28–3.16 (m, 2H), 2.97 (d, $J = 7.4$ Hz, 2H), 2.85–2.69 (m, 2H), 2.28 (s, 3H), 2.33–2.17 (m, 1H), 1.76–1.65 (m, 2H), 1.61–1.45 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 145.4, 131.1, 115.6, 70.3, 58.9, 44.9, 43.0, 31.8, 30.5, 28.1, 9.7; MS *m/z* 238.3 ($M+H^+$).

*4.1.2.6. 1-(2-Aminoethyl)-1-benzyl-5-methyl-1H-imidazole dihydrochloride (**15b**).* Dark gray solid, m. p. 227–229°C, yield 42%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.90 (br.s, 1H), 8.72 (s, 3H), 7.51–7.47 (m, 2H), 7.43 (s, 1H), 7.40–7.27 (m, 3H), 4.53 (s, 2H), 4.45 (t, $J = 6.8$ Hz, 2H), 3.18–3.05 (m, 2H), 2.31 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 146.3, 134.6, 130.9, 129.4, 129.3, 128.0, 116.2, 41.7, 37.8, 30.2, 9.6; MS *m/z* 216.3 ($M+H^+$).

*4.1.2.7. 5-Methyl-1-(4-piperidylmethyl)-2-(2-tetrahydrofuranyl)-1H-imidazole (**15c**).* Pale yellow solid, m. p. 81–83°C, yield 44%; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 6.65 (s, 1H), 4.89 (t, $J = 6.7$ Hz, 1H), 3.94 (dd, $J = 14.5$, 8.2 Hz, 1H), 3.83–3.77 (m, 2H), 3.62 (dd, $J = 14.5$, 7.0 Hz, 1H), 3.07–2.99 (m, 2H), 2.72–2.61 (m, 1H), 2.55–2.43 (m, 2H), 2.13 (s, 3H), 2.20–2.02 (m, 1H), 1.96–1.89 (m, 2H), 1.86–1.78 (m, 1H), 1.58–1.45 (m, 2H), 1.26–1.06 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 147.0, 128.7, 125.0, 72.6, 68.2, 49.1, 46.0, 37.7, 30.8, 30.7, 29.2, 26.0, 10.1; MS *m/z* 250.4 ($M+H^+$).

*4.1.2.8. 5-Methyl-2-phenyl-1-(3-pirrolidylmethyl)-1H-imidazole dihydrochloride (**15d**).* Brown solid, m. p. 204–206°C, yield 42%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.85–9.57 (m, 2H), 7.83–7.77 (m, 2H), 7.74–7.61 (m, 4H), 4.46–4.29 (m, 2H), 3.18–3.10 (m, 1H), 2.95–2.88 (m, 2H), 2.71–2.59 (m, 1H), 2.49–2.44 (m, 1H), 2.43 (s, 3H), 1.84–1.72 (m, 1H), 1.31–1.17 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 144.0, 132.4, 131.9, 130.3, 129.8, 123.6, 117.3, 46.9, 46.4, 43.8, 38.1, 27.9, 10.2; MS *m/z* 242.3 ($M+H^+$).

*4.1.2.9. 2,5-Dimethyl-1-(3-pirrolidyl)-1H-imidazole dihydrochloride (**15e**).* Dark gray solid, m. p. 42–44°C, yield 34%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.69 (br.s, 1H), 10.25 (br.s, 1H), 10.03 (br.s, 1H), 7.35 (s, 1H), 5.25 (p, $J = 9.1$ Hz, 1H), 3.81–3.67 (m, 2H), 3.52–3.42 (m, 2H), 3.29–3.19 (m, 1H), 2.73 (s, 3H), 2.59–2.52 (m, 1H), 2.39 (s, 3H), 2.37–2.29 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 145.4, 130.5, 116.1, 54.2, 45.3, 44.3, 28.7, 12.7, 11.2; MS *m/z* 166.2 ($M+H^+$).

*4.1.2.10. 5-Methyl-1-[2-(4-piperidyl)ethyl]-2-(3-pyridyl)-1H-imidazole trihydrochloride (**15f**).* Brown solid, m. p. 202–204°C, yield 41%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.38 (d, J = 9.0 Hz, 1H), 9.14 (d, J = 9.6 Hz, 1H), 9.05 (d, J = 1.6 Hz, 1H), 8.95 (d, J = 4.2 Hz, 1H), 8.41 (d, J = 8.1 Hz, 1H), 7.84 (dd, J = 7.9, 5.1 Hz, 1H), 7.71 (s, 1H), 4.19–4.09 (m, 2H), 3.13–3.05 (m, 2H), 2.67 (dd, J = 22.1, 10.8 Hz, 2H), 2.43 (s, 3H), 1.65–1.54 (m, J = 12.8 Hz, 4H), 1.48–1.40 (m, 1H), 1.35–1.21 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 150.1, 148.0, 141.4, 140.3, 132.3, 125.7, 121.3, 117.9, 43.2, 42.9, 35.3, 30.9, 28.1, 9.8; MS m/z 271.4 (M+H$^+$).

*4.1.2.11. 2,5-Dimethyl-1-(3-piperidyl)-1H-imidazole dihydrochloride (**15g**).* Brown solid, m. p. 213–215°C (decomp.), yield 41%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 14.73 (br.s, 1H), 10.01 (br.s, 2H), 7.33 (s, 1H), 5.01–4.88 (m, 1H), 3.48–3.36 (m, 2H), 3.30–3.22 (m, 1H), 3.12–3.02 (m, 1H), 2.72 (s, 3H), 2.37 (s, 3H), 2.29–2.09 (m, 2H), 2.00–1.92 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 145.0, 130.5, 116.0, 51.8, 44.7, 42.5, 26.7, 22.0, 12.7, 11.2; MS m/z 180.3 (M+H$^+$).

*4.1.3. General procedure for the preparation of compounds **9a-e** and **10a-g** exemplified by the synthesis of 4-(2,5-dimethyl-1H-imidazol-1-yl)-1-[(5-nitrofuran-2-yl)carbonyl]piperidine (**10a**)*

To a solution of 5-nitro-2-furoic acid (0.2 g, 1.27 mmol) in DMF (10 mL) CDI (0.23 g, 1.4 mmol) was added and the mixture was stirred at r. t. for 30 min. This solution was added dropwise to a mixture of **15a** (0.35 g, 1.4 mmol) and triethylamine (0.21 mL, 1.52 mmol) in DMF (15 mL) and the stirring continued for 18 h. The resulting mixture was poured into water and extracted with ethyl acetate. The organic phase was successively washed with 10% aqueous K2CO3 (2 × 10 mL), dried over anhydrous Na2SO4, filtered and concentrated *in vacuo*. The residue was suspended in diethyl ether and filtered under vacuum to obtain a beige solid: m. p. 178–180°C. Yield 47%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.76 (d, J = 3.8 Hz, 1H), 7.32 (d, J = 3.8 Hz, 1H), 6.45 (s, 1H), 4.61–4.46 (m, 1H), 4.42–4.26 (m, 2H), 3.55–3.38 (m, 2H), 3.12–2.93 (m, 1H), 2.34 (s, 3H), 2.21 (s, 3H), 2.14–1.98 (m, 2H), 1.96–1.84 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.8, 151.2, 147.7, 143.5, 126.7, 124.8, 117.0, 112.9, 53.0, 46.1, 42.1, 31.2, 30.5, 14.9, 11.2; HRMS (ESI), m/z calcd for $C_{15}H_{18}N_4O_4$ [M+H$^+$] 319.1401, found 319.1408.

*4.1.3.1. 1-(Cyclopropylmethyl)-5-methyl-2-({1-[(5-nitrofuran-2-yl) carbonyl]pyrrolidin-3-yl}methyl)-1H-imidazole (**9a**).* Brown solid, m. p. 122–124°C, yield 66%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.78–7.74 (m, 1H), 7.33 (dd, J = 11.3, 3.9 Hz, 1H), 6.53 (d, J = 6.7 Hz, 1H), 4.10–3.89 (m, 1H), 3.84–3.60 (m, 3H), 3.57–3.41 (m, 1H), 3.28–3.19 (m, 1H), 2.812.59 (m, 3H), 2.25–2.04 (m, 4H), 1.85–1.59 (m, 1H), 1.14–0.96 (m, 1H), 0.55–0.44 (m, 2H), 0.36–0.25 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 155.4, 155.3, 151.3, 148.4, 145.3, 145.2, 126.6, 126.5, 124.3, 117.3, 117.2, 113.1, 52.8, 52.3, 47.0, 46.4, 46.3, 37.7, 35.0, 31.6, 29.4, 29.3, 29.1, 11.7, 11.7, 9.7, 3.7; HRMS (ESI), m/z calcd for $C_{18}H_{22}N_4O_4$ [M+H$^+$] 359.1714, found 359.1720.

*4.1.3.2. N-[2-(1-Cyclobutyl-5-methyl-1H-imidazol-2-yl)ethyl]-5-nitrofuran-2-carboxamide (**9b**).* Brown solid, m. p. 156–158°C, yield 64%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.98 (t, J = 5.7 Hz, 1H), 7.75 (d, J = 3.9 Hz, 1H), 7.39 (d, J = 3.9 Hz, 1H), 6.50 (s, 1H), 4.77–4.62 (m, 1H), 3.57 (q, J = 7.0 Hz, 2H), 2.91 (t, J = 7.4 Hz, 2H), 2.61–2.51 (m, 2H), 2.47–2.35 (m, 2H), 2.25 (s, 3H), 1.88–1.69 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.1, 151.4, 148.3, 144.9, 127.6, 125.3, 115.7, 113.6, 49.0, 37.8, 30.0, 27.8, 14.8, 11.2; HRMS (ESI), m/z calcd for $C_{15}H_{18}N_4O_4$ [M+H$^+$] 319.1401, found 319.1395; HRMS (ESI), m/z calcd for $C_{15}H_{18}N_4O_4$ [M+Na$^+$] 341.1220, found 341.1222.

*4.1.3.3. N-[2-(1-Cyclooctyl-5-methyl-1H-imidazol-2-yl)ethyl]-5-nitrofuran-2-carboxamide (**9c**).* Beige solid, m. p. 188–190°C, yield 48%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.05 (br.s, 1H), 7.75 (d, J = 3.9 Hz, 1H), 7.39 (d, J = 3.8 Hz, 1H), 6.51 (s, 1H), 4.31–4.16 (m, 1H), 3.56 (q, J = 6.6 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H), 2.19 (s, 3H), 2.06–1.97 (m, 2H), 1.80–1.65 (m, 7H), 1.59–1.47 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.1, 151.4, 148.3, 143.9, 126.1, 115.7, 115.6, 113.6, 73.1, 55.5, 38.0, 35.6, 34.6, 34.0, 27.5, 25.8, 25.6, 25.1, 11.2; HRMS (ESI), m/z calcd for $C_{19}H_{26}N_4O_4$ [M+H$^+$] 375.2027, found 375.2040; HRMS (ESI), m/z calcd for $C_{19}H_{26}N_4O_4$ [M+Na$^+$] 397.1846, found 397.1864; HRMS (ESI), m/z calcd for $C_{19}H_{26}N_4O_4$ [M+K$^+$] 413.2586, found 413.2672.

*4.1.3.4. 5-Methyl-2-{1-[(5-nitrofuran-2-yl)carbonyl]azetidin-3-yl}-1-propyl-1H-imidazole (**9d**).* Pale yellow solid, m. p. 112–114°C, yield 42%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.76 (d, J = 3.9 Hz, 1H), 7.35 (d, J = 3.9 Hz, 1H), 6.62 (s, 1H), 4.91–4.82 (m, 1H), 4.73–4.67 (m, 1H), 4.47–4.38 (m, 1H), 4.23–4.16 (m, 1H), 4.15–4.06 (m, 1H), 3.75 (t, J = 7.5 Hz, 2H), 2.15 (s, 3H), 1.62–1.47 (m, 2H), 0.85 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.5, 151.8, 147.5, 146.4, 128.0, 124.8, 117.3, 113.3, 57.5, 54.0, 44.2, 26.2, 23.9, 11.1, 9.6; HRMS (ESI), m/z calcd for $C_{15}H_{18}N_4O_4$ [M+H$^+$] 319.1401, found 319.1394.

*4.1.3.5. 4-[(1-Methoxyethyl-5-methyl-1H-imidazol-1-yl)methyl]-1-[(5-nitrofuran-2-yl)carbonyl]piperidine (**9e**).* Orange oil, yield 21%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.75 (d, J = 3.7 Hz, 1H), 7.22 (d, J = 3.7 Hz, 1H), 6.57 (s, 1H), 4.42–4.30 (m, 1H), 4.13–4.04 (m, 1H), 4.00 (t, J = 5.1 Hz, 3H), 3.48 (t, J = 5.1 Hz, 3H), 3.40–3.34 (m, 1H), 3.21 (s, 3H), 2.88–2.77 (m, 1H), 2.58 (d, J = 6.8 Hz, 2H), 2.13 (s, 3H), 1.84–1.73 (m, 2H), 1.31–1.15 (m, 2H), 1.11–1.02 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.6, 151.2, 148.2, 145.7, 127.2, 123.2, 116.5, 113.0, 71.2, 58.4, 46.6, 42.7, 42.6, 34.4, 32.4, 31.3, 9.5; HRMS (ESI), m/z calcd for $C_{18}H_{24}N_4O_5$ [M+H$^+$] 377.1819, found 377.1815.

*4.1.3.6. N-[2-(2-Benzyl-5-methyl-1H-imidazol-1-yl)ethyl]-5-nitrofuran-2-carboxamide (**9f**).* Brown solid, m. p. 94–96°C, yield 62%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.05 (t, J = 5.7 Hz, 1H), 7.74 (d, J = 3.9 Hz, 1H), 7.38 (d, J = 3.9 Hz, 1H), 7.29–7.16 (m, 5H), 6.57 (s, 1H), 4.02 (s, 2H), 3.94 (t, J = 6.8 Hz, 2H), 3.43–3.35 (m, 2H), 2.16 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.5, 151.4, 147.3, 145.7, 138.0, 128.4, 128.3, 127.2, 126.2, 124.5, 115.8, 113.4, 41.7, 39.0, 32.6, 9.3; HRMS (ESI), m/z calcd for $C_{18}H_{18}N_4O_4$ [M+H$^+$] 355.1401, found 355.1394.

*4.1.3.7. N-[2-(2-Benzyl-5-methyl-1H-imidazol-1-yl)ethyl]-5-nitrofuran-2-carboxamide (**10b**).* Brown solid, m. p. 94–96°C, yield 62%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.05 (t, J = 5.7 Hz, 1H), 7.74 (d, J = 3.9 Hz, 1H), 7.38 (d, J = 3.9 Hz, 1H), 7.29–7.16 (m, 5H), 6.57 (s, 1H), 4.02 (s, 2H), 3.94 (t, J = 6.8 Hz, 2H), 3.43–3.35 (m, 2H), 2.16 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.5, 151.4, 147.3, 145.7, 138.0, 128.4, 128.3, 127.2, 126.2, 124.5, 115.8, 113.4, 41.7, 39.0, 32.6, 9.3; HRMS (ESI), m/z calcd for $C_{18}H_{18}N_4O_4$ [M+H$^+$] 355.1401, found 355.1394.

*4.1.3.8. 4-[(5-Methyl-2-tetrahydrofuran-2-yl)-1H-imidazol-1-yl) methyl]-1-[(5-nitrofuran-2-yl)carbonyl]piperidine (**10c**).* Pale brown solid, m. p. 91–93°C, yield 28%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.75 (d, J = 3.9 Hz, 1H), 7.23 (d, J = 3.9 Hz, 1H), 6.59 (s, 1H), 4.96 (t, J = 6.7 Hz, 1H), 4.47–4.31 (m, 1H), 4.19–4.07 (m, 1H), 3.97–3.82 (m, 2H), 3.82–3.67 (m, 3H), 3.19–3.07 (m, 1H), 2.86–2.69 (m, 1H), 2.57–2.44 (m, 3H), 2.17 (s, 3H), 2.11–1.94 (m, 5H), 1.93–1.83 (m, 1H), 1.66–1.56 (m, 1H), 1.55–1.46 (m, 1H), 1.36–1.21 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.7, 151.2, 148.0, 146.7, 128.6, 124.1, 116.6, 113.0, 71.7, 67.5, 47.6, 46.3, 42.2, 36.6, 29.9, 28.9, 25.7, 9.7; HRMS (ESI), m/z calcd for $C_{19}H_{24}N_4O_5$ [M+H$^+$] 389.1819, found 389.1813; HRMS (ESI), m/z calcd for $C_{19}H_{24}N_4O_5$

[M+Na$^+$] 411.1639, found 411.1639.

*4.1.3.9. 5-Methyl-1-({1-[(5-nitrofuran-2-yl)carbonyl]pyrrolidin-3-yl} methyl)-2-phenyl-1H-imidazole (**10d**).* Brown solid, m. p. 129–131 °C, yield 48%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.75 (dd, J = 5.2, 4.1 Hz, 1H), 7.59–7.50 (m, 2H), 7.46–7.34 (m, 3H), 7.19 (dd, J = 23.4, 3.9 Hz, 1H), 6.79 (d, J = 3.5 Hz, 1H), 4.19–4.08 (m, 2H), 3.72–3.43 (m, 3H), 3.24–2.95 (m, 1H), 2.49–2.33 (m, 1H), 2.27 (s, 3H), 1.86–1.68 (m, 1H), 1.52–1.36 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 203.8, 155.3, 155.2, 151.2, 148.1, 148.1, 146.7, 146.6, 131.8, 131.7, 128.8, 128.6, 128.6, 128.5, 128.3, 128.2, 126.2, 126.1, 117.4, 117.3, 113.1, 113.0, 50.2, 49.5, 46.2, 45.8, 44.9, 44.8, 36.9, 29.0, 26.4, 9.9, 9.8; HRMS (ESI), *m/z* calcd for $C_{20}H_{20}N_4O_4$ [M+H$^+$] 381.1557, found 381.1547.

*4.1.3.10. 2,5-Dimethyl-1-{1-[(5-nitrofuran-2-yl)carbonyl]pyrrolidin-3-yl}-1H-imidazole (**10e**).* Dark orange solid, m. p. 84–86 °C, yield 57%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.80 (dd, J = 7.3, 3.9 Hz, 1H), 7.44 (dd, J = 5.9, 4.0 Hz, 1H), 6.53 (br.s, 1H), 5.04–4.91 (m, 1H), 4.30–4.12 (m, 1H), 4.04–3.94 (m, 1H), 3.93–3.81 (m, 1H), 3.80–3.65 (m, 1H), 2.45–2.31 (m, 5H), 2.23 (d, J = 4.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 155.7, 155.7, 151.4, 147.9, 147.9, 144.2, 144.1, 127.2, 127.1, 125.0, 124.8, 117.7, 117.7, 113.06, 53.8, 51.9, 49.6, 49.0, 46.4, 45.4, 30.5, 27.3, 14.6, 14.5, 10.9, 10.8; HRMS (ESI), *m/z* calcd for $C_{14}H_{16}N_4O_4$ [M+H$^+$] 305.1244, found 305.1256; HRMS (ESI), *m/z* calcd for $C_{14}H_{16}N_4O_4$ [M+Na$^+$] 327.1064, found 327.1080.

*4.1.3.11. 4-[(5-Methyl-2-(3-pyridyl)-1H-imidazol-1-yl)ethyl]-1-[(5-nitrofuran-2-yl)carbonyl]piperidine (**10f**).* Brown solid, m. p. 79–81 °C, yield 38%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.78 (s, 1H), 8.62 (d, J = 4.2 Hz, 1H), 7.99 (d, J = 7.7 Hz, 1H), 7.73 (d, J = 3.6 Hz, 1H), 7.51 (dd, J = 7.5, 5.0 Hz, 1H), 7.20 (d, J = 3.7 Hz, 1H), 6.82 (s, 1H), 4.33–4.19 (m, 1H), 4.08–3.94 (m, 3H), 3.17–3.02 (m, 1H), 2.81–2.66 (m, 1H), 2.27 (s, 3H), 1.63–1.46 (m, 5H), 1.16–1.02 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 156.6, 151.2, 149.2, 148.9, 148.1, 143.5, 135.7, 129.4, 127.7, 126.6, 123.7, 116.6, 113.0, 46.4, 42.4, 41.6, 36.4, 32.9, 32.0, 31.0, 9.5; HRMS (ESI), *m/z* calcd for $C_{21}H_{23}N_5O_4$ [M+H$^+$] 410.1823, found 410.1839; HRMS (ESI), *m/z* calcd for $C_{21}H_{23}N_5O_4$ [M+Na$^+$] 432.1642, found 432.2658.

*4.1.3.12. 3-(2,5-Dimethyl-1H-imidazol-1-yl)-1-[(5-nitrofuran-2-yl) carbonyl]piperidine (**10g**).* Pale brown solid, m. p. 65–67 °C, yield 49%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.76 (d, J = 5.3 Hz, 1H), 7.29 (br.s, 1H), 6.50 (s, 1H), 4.51–4.30 (m, 1H), 4.29–4.03 (m, 2H), 3.70–3.57 (m, 2H), 2.36 (s, 3H), 2.24 (s, 3H), 2.26–2.10 (m, 1H), 2.05–1.94 (m, 1H), 1.91–1.82 (m, 1H), 1.74–1.59 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 157.1, 151.3, 147.7, 143.8, 127.0, 124.6, 117.3, 113.0, 65.0, 53.0, 52.4, 49.4, 46.3, 46.0, 42.2, 29.2, 25.6, 24.2, 15.2, 14.8, 11.2; HRMS (ESI), *m/z* calcd for $C_{15}H_{18}N_4O_4$ [M+H$^+$] 319.1401, found 319.1395.

4.2. Determination of antimycobacterial activity in vitro

Mycobacterium tuberculosis H_{37}Rv strain (originated from the Institute of Hygiene and Epidemiology in Prague, 1976) was obtained on August 7th, 2013 from the Federal Scientific Center for Expertise of Medical Products (RF Ministry of Health Care). The lyophilized strain was seeded on Löwenstein–Jensen growth medium. The 3-weeks culture was suspended in physiological solution containing glycerol (15%) and transferred into cryotubes to be kept at −80 °C. Three weeks in advance of the experiment, the culture was brought to ambient temperature and re-seeded into Löwenstein–Jensen growth medium. Thus, the 2nd generation of the original *M. tuberculosis* culture was used in present study.

The minimal inhibitory concentration (MIC) of the compounds was determined using the REMA (resazurin microtitre plate assay) [36]. A 3-week *M. tuberculosis* culture was transferred into a dry, sterile tube containing 8–9 3-mm glass beads. The tube was placed on a Vortex shaker for 30–40 s and then 5 mL Middlebrook 7H9 Broth (Becton Dickinson, catalogue No. 271310) was introduced. The turbidity of bacterial suspension was adjusted to 1.0 McFarland units (corresponding to approximately $3x10^8$ bacteria/mL) and diluted 20-fold with Middlebrook 7H9 Broth containing OADC enrichment (Becton Dickinson, catalogue No. 245116). The same culture medium was used to prepare the 1:100 *M. tuberculosis* (1% population) control. The stock solutions of the compounds in DMSO (10 mg/mL) were diluted with Middlebrook 7H9 Broth (containing OADC enrichment) to a concentration of 800 μg/mL 200 μL of the solution thus obtained was introduced into the 2nd row of a 96-well microtitre plate. This raw was used to perform 2-fold serial dilutions using and 8-channel pipette to obtain final concentrations of 1.6, 3.1, 6.2, 12.5, 25, 50, 100, 200 and 400 μg/mL concentrations of the compound in rows 2–9 (accounting for 100 μL of bacterial suspension introduced for testing). Row 10 – MTb suspension control, row 11 – same culture diluted 10-fold (the 1% control). Row 12 was used as a blank control for optical density reading (200 μL of the grown medium). The bacterial suspension (100 μL) was introduced into each well except rows 11 (1% population control) and 12 (blank culture medium), to the total volume of 200 μL in each well. The plates were incubated at 35 °C for 7 days. At that point, 0.01% aqueous solution (30 μL) of resazurin (Sigma, product No. R7017) was introduced in each well and the incubation continued for 18 h at 35 °C. The fluorescence reading was performed using FLUOstar Optima plate reader operating at λ_{ex} = 520 nm and λ_{em} = 590 nm. The bacterial viability was determined by comparing the mean values (±SD at p = 0.05) of fluorescence in the control wells (row 12, blank and row 11, 1% control) and the wells containing the compound tested. The MIC was determined as the compound concentration at which the fluorescence reached a plateau or was statistically (t criterion) similar to that of 1% control.

The mutant, patient-derived multidrug-resistant strains of *M. tuberculosis* were obtained from the pathogenic mycobacterial strain collection of Saint Petersburg Research Institute of Phthisiopulmonology. These strains were genotyped using microchip technology and were confirmed to contain various combinations of mutations in genes *rpoB* (resistance to rifampicin), *katG*, *inhA* and *ahpC* (resistance to isoniazid) as shown in Table 3.

4.3. Counter-testing against non-mycobacterial strains

Testing of susceptibility of other microorganisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter aerogenes*) to compound **9d** as well as ciprofloxacin (positive control) was performed using the conventional Kirby-Bauer disk diffusion test [37] under the Standart Operating Procedure of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) [38]. Disks containing 5 μg of ciprofloxacin were used. Compound **9d** (1 mg) dissolved in dimethyl sulfoxide (10 μL) and diluted to a volume of 1 mL with deionized water. To a Petri dish containing Muller-Hilton agar inoculated with a bacterial suspension (McFarland OD = 0.5) 5 μL of this solution (containing 5 μg of compound **9d**) was added. After drying of the compound solution, the Petri dish was incubated at 37 °C for 18 h. The susceptibility to a drug was assessed by measuring the bacterial growth inhibition zone diameter around the disc with ciprofloxacin or the compound **9d** dried solution circular spot.

4.4. Toxicology studies

The experiment was conducted on 100 sexually mature male C57BL6 mice (initial body weight range 19.0–22.0 g). The animals were kept according to ETS 123 (European Convention for the Protection of Vertebrate Animals Use for Experimental and Other Scientific Purposes, Strasbourg 18. III.1986). Animals were quarantined for 14 days during which period the animals were examined on a daily basis. The mice were kept in plastic cages (15–20 animals per cage) with sawdust flooring. Daylight and dark phases were alternating each 12 h, the ambient temperature was maintained within +23…+25 °C range with humidity in 50–70% range. The air in the cages was actively circulated by a ventilation system and was disinfected by a UV lamp.

The animals were divided at random in groups of 6 mice for the treatment and one group of 7 animals to be used as a control (no treatment) group. Randomization criteria included the absence of sickness signs and group homogeneity with respect to mouse body weight. A given dose of compound **9d** was given orally via a metal gavage as a suspension in Tween-80 diluted with a calculated amount of distilled water. Animals from the control group were receiving 0.01% aqueous solution of Tween-80. Dosage precision was regulated by the variable volume of the drug solution administered (normalized to the animal's body weight) at a constant concentration of the solution. Compound **9d** dose was varied in the 700…1100 mg/kg range and increased by 100 mg/kg between the treatment groups. Each dose was administered to 6 animals.

The following parameters were recorded during the experiment: animal lethality, time to death, poisoning symptoms and the observable changes in the animals' general condition recorded daily over 14 days of treatment. Animals were euthanized by cervical dislocation. Both dead and euthanized animals were analyzed for macroscopic parameters. Surviving and euthanized animals were analyzed for the fraction of heart, lungs, liver, spleen and left kidney relative to the total body weight (see Table S3).

The median lethal dose (LD_{50}) was calculated using the following formula: $LD_{50} = LD_{100} - \Sigma$ (z,d)/m, where z is half of the total number of animals that died after the following two doses, m – number of animals receiving each dose. Standard error of measurement was calculated using the following folmula: SEM (LD_{50}) = sqrt (k·SD/n), where n = 6 (animals per group), D = 100 (dose excalation increment), $S = LD_{84} - LD_{16}$. Statistical analysis was performed using the Student's parametric *t*-test.

4.5. In vivo efficacy testing

The efficacy testing of compound **9d** against experimental tuberculosis model was conducted using 71 inbred C57black/6 mice according to the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes adopted on 22 September 2010. The tuberculosis was modeled by inoculation of mice with a suspension of three-week culture of *Mtb* (10^6 cfu per mouse). In the first series of experiments (n = 37), the same drug-sensitive H37Rv strain of *Mtb* as was used for *in vitro* compound testing (see Section 4.2) was employed. In the second series of experiments, the mice (n = 34) were inoculated with the same patient-derived strain 2712 of *Mtb* (obtained from the pathogenic mycobacterial strain collection of Saint Petersburg Research Institute of Phthisiopulmonology) with an established multidrug resistance and mutations profile as was used for *in vitro* compound testing (see Table 3).

Macroscopic pulmonary evaluation of euthanized mice (5 animals from each group) performed 10 days post inoculation demonstrated the presence of tubercular foci in the lungs. The animals for each series of experiments were randomized into three groups. The first series of experiments (H37Rv strain) included (A) the no-treatment group (n = 17), (B) the group receiving etambutol (n = 10) and (C) the group receiving compound **9d** (n = 10). Etambutol (20 mg/kg) and compound **9d** (20 mg/kg) were administered orally as suspension in water (0.2 mL/mouse) using a metal gavage over 42 days (excluding weekends). The second series of experiments (2712 strain) included (A) the no-treatment group (n = 15), (B) the group receiving the efficacious combination of prothionamide (12.5 mg/kg), levofloxacin (12.5 mg/kg), *p*-aminosalicylic acid (130 mg/kg) and cycloserine (12.5 mg/kg) to which 2712 strain was found susceptible *in vitro* (n = 6) and (C) the group receiving the same combination of drugs except for cycloserine which was substituted for compound **9d** (28 mg/kg, i. e. 1/10 LD_{16}). The drugs were administered orally as suspension in water (0.2 mL/mouse) using a metal gavage over 3 months (excluding weekends).

The animals were euthanized by cervical dislocation. The therapeutic efficacy in the treatment groups were compared with that in the no-treatment group by lung mass indices (in relative units), tubercular foci indices (in relative units) and by cfu per lung mass unit count (determined after seeding a given quantity of the lung homogenate on the Löwenstein–Jensen medium). The data were analyzed using the Statistica 7.0 software package employing and evaluated for significance using the Student's t-test.

Acknowledgements

This research was supported by the Russian Science Foundation (project grant 14-50-00069). Mass spectrometry studies were performed at the Center for Chemical Analysis and Materials Research of the Saint Petersburg State University Research Park.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.08.068.

References

[1] J.M.A. Blair, M.A. Webber, A.J. Baylay, D.O. Ogbolu, L.J.V. Piddock, Nat. Rev. Microbiol. 13 (2005) 42–51.
[2] J.R. Corey, M.E. Stryjewski, W. Weyenberg, U. Yasothan, P. Kirkpatrick, Nat. Rev. Drug Discov. 8 (2009) 929–930.
[3] D.M. Barter, S.O. Agboola, M.B. Murray, T. Bärnighausen, BMC Publ. Health 12 (2012) 980.
[4] D.H. Diacon, A. Pym, M. Grobusch, et al., N. Engl. J. Med. 360 (2009) 2397–2405.
[5] M. Stehr, A.A. Elamin, M. Singh, Curr. Top. Med. Chem. 14 (2014) 110–129.
[6] R.C. Goldman, Future Med. Chem. 2 (2010) 1253–1271.
[7] L. Zhou, H. Ishizaki, M. Spitzer, K.L. Taylor, N.D. Temperley, S.L. Johnson, P. Brear, P. Gautier, Z. Zeng, A. Mitchell, V. Narayan, E.M. McNeil, D.W. Melton, T.K. Smith, M. Tyers, N.J. Westwood, E.E. Patton, Chem. Biol. 19 (2012) 883–892.
[8] V. Purohir, A.K. Basu, Chem. Res. Toxicol. 13 (2000) 673–692.
[9] M. Hofnung, P. Quillardet, V. Michel, E. Touati, Res. Microbiol. 153 (2002) 427–434.
[10] O. Dann, E.F. Möller, Chem. Ber. 80 (1947) 23–36.
[11] R.E. Chamberlain, J. Antimicrob. Ther. 2 (1976) 325–336.
[12] C.K. Stover, P. Warrener, D.R. VanDevanter, D.R. Sherman, T.M. Arain, M.H. Langhorne, S.W. Anderson, J.A. Towell, Y. Yuan, D.N. McMurray, B.N. Kreiswirth, C.E. Barry, W.R. Baker, Nature 405 (2000) 962–966.
[13] A. Huttner, E.M. Verhaegh, S. Harbarth, A.E. Muller, U. Theuretzbacher, J.W. Mouton, J. Antimicrob. Chemother. 70 (2015) 2456–2464.
[14] S. Ekins, R. Pottorf, R.C. Reynolds, A.J. Williams, A.M. Clark, J.S. Freundlich, J. Chem. Inf. Model. 54 (2014) 1070–1082.
[15] R.P. Tangallapally, R. Yendapally, A.J. Daniels, R.E.B. Lee, R.E. Lee, Curr. Top. Med. Chem. 7 (2007) 509–526.
[16] J.G. Hurdle, R.B. Lee, N.R. Budha, et al., J. Antimicrob. Chemother. 62 (2008) 1037–1045.
[17] M. Krasavin, V. Parchinsky, G. Kantin, O. Manicheva, M. Dogonadze, T. Vinogradova, B. Karge, M. Brönstrup, Bioorg. Med. Chem. 25 (2017) 1867–1874.
[18] P. Zheng, S. Somersan-Karakaya, S. Lu, J. Roberts, M. Pingle, T. Warrier, D. Little, X. Guo, S.J. Brickner, C.F. Nathan, B. Gold, G. Liu, J. Med. Chem. 57

(2014) 3755–3772.
[19] K. Ran, C. Gao, H. Deng, Q. Lei, X. You, N. Wang, Y. Shi, Z. Liu, W. Wei, C. Peng, L. Xiong, K. Xiao, L. Yu, Bioorg. Med. Chem. Lett 26 (2016) 3669–3674.
[20] Y.-L. Fan, X.-H. Jin, Z.-P. Huang, H.-F. Yu, Z.-G. Zeng, T. Gao, L.-S. Feng, Eur. J. Med. Chem. 150 (2018) 347–365.
[21] A. Trapero, A. Pacitto, V. Singh, M. Sabbah, A.G. Coyne, V. Mizrahi, T.L. Blundell, D.B. Ascher, C. Abell, J. Med. Chem. 61 (2018) 2806–2822.
[22] A. Pews-Davtyan, M. Beller, Chem. Commun. 47 (2011) 2152–2154.
[23] A. Safrygin, E. Krivosheyeva, D. Dar'in, M. Krasavin, Synthesis 50 (2018) (in press), https://doi.org/10.1055/s-0036-1591599.
[24] R.P. Tangallapally, R. Yendapally, R.E. Lee, K. Hevener, V.C. Jones, A.J.M. Lenaerts, M.R. McNeil, Y. Wang, S. Franzblau, R.E. Lee, J. Med. Chem. 47 (2004) 5276–5283.
[25] G. Vistoli, A. Pedretti, B. Testa, Drug Discov. Today 13 (2008) 285–294.
[26] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Adv. Drug Deliv. Rev. 46 (2001) 3–26.
[27] T.D. Davis, C.J. Gerry, D.S. Tan, ACS Chem. Biol. 9 (2014) 2535–2544.
[28] G.R. Bickerton, G.V. Paolini, J. Besnard, S. Muresan, A.L. Hopkins, Nat. Chem. 4 (2012) 90–98.
[29] J.A. Karlowsky, D.J. Hoban, M.A. Hackel, S.H. Lob, D.F. Sahm, J. Med. Microbiol. 66 (2017) 61–69.
[30] H.C. Hodge, L.H. Sterner, Clinical Toxicology of Commercial Products. Acute Poisoning. 4th Edition. Baltimore, 1975, 427 pp.
[31] S.D. Ahuja, D. Ashkin, M. Avendano, R. Banerjee, M. Bauer, J.N. Bayona, et al., PLoS Med. 9 (2012) e1001300.
[32] In-house Data of Saint Petersburg Research Institute of Phthisiopulmonology.
[33] Treatment of Tuberculosis, Guidelines. 4th Edition. Geneva, World Health Organization, 2010 (Chapter 7): Treatment of drug-resistant tuberculosis.
[34] J. Chen, S. Zhang, P. Cui, W. Shi, W. Zhang, Y. Zhang, J. Antimicrob. Chemother. 72 (2017) 3272–3276.
[35] S.R. Snavely, G.R. Hodges, Ann. Intern. Med. 101 (1984) 92–104.
[36] A. Martin, M. Camacho, F. Portaels, C.C. Palomino, Antimicrob. Agents Chemother. 47 (2003) 3616–3619.
[37] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Am. J. Clin. Pathol. 45 (1966) 493–496.
[38] Standard Operating Procedure: Procedure for establishing zone diameter breakpoints and quality control criteria for new antimicrobial agents, EUCAST SOP 9 (2018) 1.